REMARKS

Claims 1-35 are now pending. Claims 1 and 26 are currently amended. Claims 3, 5, 6, 9, 11, 12, 15, 17, 18, 25, 28, and 30-32 have been previously amended. Claims 33-35 have been previously withdrawn. Reconsideration of the application, as amended, is requested in view of the remarks below.

General objections to the Specification

The Examiner requested that embedded hyperlink be removed from the Specification. See the Office Action, page 2, lines 6-9. Applicants have replaced the embedded hyperlink with plain texts.

The Examiner also pointed out that trademarks have not been capitalized and accompanied by generic terminology. See the Office Action, page 2, lines 10-12. Applicants have corrected this informality by capitalizing all trademarks and adding generic terminology (e.g., a GENE PULSER electroporation apparatus and a HIPREP 16/10 Octyl FF chromatographic column).

Finally, The Examiner objected to the Specification in view of the misalignment of nucleic acid and amino acid sequences. See the Office Action, page 2, lines 16-20. Applicants have amended SEQ ID NO:1 through 10 such that the nucleic acid sequences are now properly aligned with their corresponding amino acid sequences. The amended sequence alignment would allow the identification of mutated nucleic acids (in darkened background) with mutated amino acids (in bold face). For clarity, Applicants also underline all N-terminal peptides.

Rejection under 37 CFR 1.71

The Examiner objected to the Specification under 37 CFR 1.71 in view of the discrepancy found between the sequence listing and the sequence alignment. See the Office Action, page 2, lines 21-31. Applicants have submitted an amended sequence listing that is consistent with the sequence alignment disclosed in the Specification. This amended sequence

listing, which adds no new matter, would allow the Examiner to conduct a prior art search on claims 25 and 32.

Rejection under 37 CFR 1.75(c)

The Examiner objected to each of claims 6, 12, 18, and 32 under 37 CFR 1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. Specifically, The Examiner alleged that the objected claims are drawn to a mutant *C. rugosa* lipase of SEQ ID NO:4, which is not a wild-type sequence as suggested by the Specification on page 14, lines 6-8. See the Office Action, page 2, lines 32-35, and page 3, lines 1-5. Applicants have identified a spelling mistake at page 14, line 7 in the Specification (i.e., ...differs form the wild-type *C. rugosa* lipase 3...), that might have misled the Examiner to incorrectly interpret the identity of SEQ ID NO:4. Applicants have replaced 'form' with 'from' to precisely point out that SEQ ID NO:4 is indeed a mutant amino acid sequence which differs from the wild-type sequence. Therefore, claims 6, 12, 18, and 32, all drawing to a mutant *C. rugosa* lipase of SEQ ID NO:4, are properly dependent on claim 1, which is limited to a mutant *C. rugosa* DNA sequence. Applicants assert that the rejection under CFR 1.75(c) has been overcome based on the remarks set froth above.

Rejection under U.S.C. 112, second paragraph

(1) The Examiner asserted that the clause "includes at least 12 codons corresponding to CTG codons in the wild-type DNA, each of the 12 codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC" renders claim 1 indefinite and confusing. See the Office Action, page 3, lines 13-18. Applicants have replaced the clause in claim 1 with "includes at least 12 codons substituted for the CTG codon in the wild-type DNA, each of the 12 codons, independently, being TCT, TCC, TCA, TCG, AGT, or AGC."

(2) The Examiner argued that the clause "wherein the amino acid sequence of the *Candida rugosa* lipase is SEQ ID NO: 4" in claims 6, 12, 18, and 32 renders the claims indefinite and confusing for reasons set forth in the rejection under CFR 1.75(c). See the Office Action, page

3, lines 19-22. Applicants have amended the Specification at page 14, lines 6-8 to particularly point out and distinctly claim the subject matter, i.e., a mutant *C. rugosa* lipase of SEQ ID NO:4. In view of this amendment, Applicants submit that claims 6, 12, 18, and 32 are now in condition for allowance.

(3) The Examiner asserted that the phrase "correspond to at least [12] CTG codons" in claim 26 renders the claim indefinite and confusing. See the Office Action, page 3, lines 23-25. Applicants have replaced the phrase with "substitute for at least 12 CTG codons."

(4) The Examiner pointed out that the number of nucleotides in SEQ ID NO: 3 in the sequence listing does not match with that in the Specification on pages 6 and 7. See the Office Action, page 3, lines 26-28. Applicants have amended the sequence listing such that the number of nucleotides in SEQ ID NO:3 match with that in the specification.

The Examiner also pointed out that SEQ ID NO:3 in the sequence listing does not encode SEQ ID NO:4, thereby rendering claims 25 and 32 indefinite and confusing. See the Office Action, page 3, lines 28-30. Applicants have amended the sequence listing such that the nucleotide of SEQ ID NO:3 encodes the polypeptide of SEQ ID NO:4.

(5) The Examiner rejected claims 5, 7, 9, 11, 13, 15, 17, 19-24, 30, and 31 for being dependent on rejected claims. See the Office Action, page 3, lines 31-33. Applicants submit that amended claims 1 and 26 are now in condition for allowance. For the same reason, all the other pending claims, which depend from amended claims 1 and 26 either directly or indirectly, are also believed to be in condition for allowance.

Rejection under 35 §U.S.C. 103(a)

The Examiner rejected claims 1-5, 7-11, 13-17, 18-24, and 26-30 as being obvious over Bocca *et al.* or WO 99/14338 ("WO '388") in view of Lotti *et al.* ("Lotti") and Ge *et al.* ("Ge"). See the Office Action, page 4, lines 8-11. Note that all the authors of Bocca *et al.* are named as inventors in WO '388. The subject matter disclosed in these two references are essentially identical. For this reason, Applicants use the term "Bocca" below when referring to both Bocca *et al.* and WO '388.

A brief discussion on gene expression in *C. rugosa* is believed to be in order. *C. rugosa* obeys unusual codon usage in which the CTG codon, a universal codon for leucine, is read as serine. Thus, when expressing a *C. rugosa* gene in a heterologous host (e.g., *S. cerevisiae* and *P. pastoris*), the CTG codon is read as leucine instead of serine, and it is necessary to "restore" the serine residues by replacing CTG codons with other universal codons for serine, i.e., TCT, TCC, TCA, TCG, AGT, or AGC.

Claim 1, as amended, is drawn to a mutant *C. rugosa* lipase (CRL) gene, which contains **at least 12** universal serine condons substituted for the CTG codons in the wild-type and is **at least 80%** identical to a wild-type CRL gene.

According to the Examiner, Bocca teaches constructing "mutated LIP1 genes [which encode *C. rugosa* lipase 1] by site directed mutagenesis in which 2-**8** CUG codon are substituted with universal serine codons, ... Also, they constructed a synthetic gene in which all the 19 CTGs codons are substituted with universal serine codons and the substitution of uncommon codons with common codons for *S. cerevisiae* and *P. pastoris*...." See the Office Action, page 4, lines 17-23; emphasis added.

Indeed, Bocca discloses two types of CRL constructs for expression in *S. cerevisiae*. One type involves mutant sequences which included up to **8** universal serine condons substituted for CTG codons in the wild-type. See Bocca *et al.*, page 1416, Table 1. Another type involves a synthetic gene which had undergone three genetic modifications: [1] all universal serine condons were substituted for CTG codons in the wild-type, [2] uncommon codons, not related to serine codons, that are rarely represented in *S. cerevisiae* were substituted with common ones, and [3] unique restriction sites were strategically positioned throughout the gene. See Bocca *et al.*, page 1416, right column, last four lines.

As mentioned above, claim 1 is drawn to a mutant CRL gene, which contains **at least 12** universal serine codons substituted for CTG codons in the wild-type. The first type of Bocca constructs, made by site-directed mutagenesis, only includes up to **8** universal serine codons substituted for CTG codons in the wild-type, not **at least 12**. Of note, this construct failed to produce a functional CRL protein. See WO '388, page 3, lines 32-38; and Bocca *et al.*, page

1416, right column, paragraph 3. By contrast, the CRL genes covered by claim 1, having at least 12 CTG replacement codons, produce active proteins. See Table I-V in the Specification. In other words, Applicants have found out that at least 12 CTG replacement codons are critical to produce active lipases, which is not taught or suggested in Bocca.

Clearly, Bocca *et al.* were not successful in making a construct which contains more than 8 universal serine codons substituted for CTG codons in the wild-type, as it was very difficult to make such a construct by site-directed mutagenesis. According to WO '388, "the task of undertaking such large scale mutagenesis is tremendous i.e.[,] an awful lot of effort with very little expectation of success." See page 4, lines 12-13. According to Bocca *et al.*, changing all CTG codons of a CRL gene is "a tremendous task." See page 1416, left paragraph, paragraph 5.

Bocca *et al.* proposed to prepare the second type of constructs. This construct is a synthetic gene which had undergone the three above-mentioned genetic modifications. It produced a functional CRL protein. Obviously, Bocca *et al.* believed that merely substituting CTG codons (i.e., modification [1]) was not adequate to produce a functional CRL protein, as evidenced by their endeavor to also convert uncommon codons to common ones (modification [2]). The approach taken by Bocca *et al.* necessitated introduction of unique restriction sites (modification [3]). As a result of these three genetic modifications, the second type of Bocca constructs is only **77%** identical to its wild-type counterpart. See Bocca *et al.*, page 1417, left column, lines 2-4. By contrast, claim 1, drawn to a mutant CRL gene having at least 80% sequence identity, covers a mutant CRL3 gene (SEQ ID: 3) which is **93.7%** identical to its wild-type counterpart, and sequence identity of other exemplary mutant CRL proteins, i.e., CRL2, 4, 5, and 8, are determined to be 94%, 94%, 94%, and 92%, respectively. See the Specification, pages 5-12. Indeed, high sequence identity, i.e., at least 80% as recited in claim 1, is a reflection of only conducting CTG codon substitution (i.e., modification [1]), but not modifications [2] and [3].

The teachings of Bocca, therefore, discourage one skilled artisan from making a mutant CRL gene which contains more than 8 universal serine codons substituted for CTG codons in the wild-type by site-directed mutagenesis. Rather, they encourage people to adopt the expensive

and elaborate synthetic method and include modifications [1], [2], and [3] to the synthetic gene. To the extent that Bocca teaches modifications [1], [2], and [3], it teaches away from what Applicants have attempted and successfully accomplished, i.e., modifying a wild-type only by substituting 12 or more universal serine codons for CTG codons.

The Examiner also cited Lotti and Ge. Lotti teaches molecular cloning and characterization of three CRL genes without mentioning expression of a functional CRL protein. See page 45, the Summary. In other words, Lotti does not teach replacement of CTG codons at all. Ge teaches a site-directed mutagenesis procedure to simultaneously introduce 3 cat- and 6 lacI-related mutations. See page 28, Figure 1. Ge does not teach simultaneously introducing at least 12 mutations to a gene. The mutations disclosed in Ge are not in any way related to CTG codons. In other words, Lotti and Ge are entirely irrelevant to claim 1, or claims dependent from it, all of which are drawn to a mutant CRL gene in which at least 12 universal serine codons are substituted for CTG codons in the wild-type.

For the reasons set forth above, Applicants submit that claim 1 is not rendered obvious by Bocca, Lotti, and Ge, in any combination. Neither are claims 2-5, 7-11, 13-17, and 18-24, all of which depend either directly or indirectly from claim 1.

Claim 26 is drawn to a method of preparing a mutant CRL gene by conducting PCR amplification to **simultaneously** introduce **at least 12** universal serine codons substituted for CTG codons in the wild-type. As discussed above, Bocca *et al.* proposed two types of constructs. For the first type, mutations were introduced one by one, not simultaneously. For the second type, the gene was synthesized de novo, instead of by site-directed mutagenesis of a wild-type gene. Lotti, focusing on cloning three CRL genes, does not teach or suggest substituting universal serine condons substituted for CTG codons in the wild-type genes. Finally, Ge discloses a mutagenesis method to introduce up to 6 mutations simultaneously. No where in Ge is it suggested that one can simultaneously introduce at least 12 mutations, **twice** that taught in Ge. Claim 26 is therefore not rendered obvious by any combination of Bocca, Lotti, and Ge. Neither are claims 27-30, all of which depend from claim 26.

The Examiner also objected to claims 6, 12, 18, and 31 as being dependent upon a rejected base claim. See the Office Action, page 6, lines 1-2. Applicants believe that all of the rejected base claims are now in condition for allowance and this objection should be withdrawn.

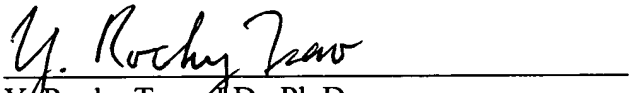
CONCLUSION

Applicants believe that all claims, as amended, cover allowable subject matter. Early allowance by the Examiner is respectfully solicited.

Please apply any other charges to deposit account 06-1050, referencing attorney docket 15664-002US1.

Respectfully submitted,

Date: 2-14-05



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